THE DEVELOPMENT OF RECIPROCAL ANDROGENETIC FROG HYBRIDS [1]

ANNA-BETTY CLARK MOORE

Department of Zoology, Columbia University, New York City

The role of the cytoplasm in development has long been of interest to embryologists. As early as 1898, Driesch described echinoderm hybrids as purely maternal and concluded that early development is determined by the egg cytoplasm. Conklin (1908) wrote that early development, which includes polarity, symmetry, type of cleavage, relative positions and proportions of future organs, is predetermined in the cytoplasm of the egg. Conklin pointed out that these differentiations arise during ovarian history as a result of the inter-action of the nucleus and the cytoplasm, and thus both play a part in the predetermination of the cytoplasm. These conclusions were based by Conklin on his study of Ascidian eggs (1905) and Wilson's studies of Dentalium and Patella (1904).

One approach to the question of the role of the cytoplasm in development has been the study of amphibian embryos obtained by fertilizing, with foreign sperm, eggs whose maternal chromosomes have been inactivated or removed. P. Hertwig (1916) was one of the earliest workers in this field but it was Baltzer (1920) who initiated the great bulk of this work, using androgenetic Triturus hybrids. These developmental studies have demonstrated the major role of the nucleus in development but the part played by the cytoplasm has not been clarified. I believe that one reason for the failure to demonstrate the cytoplasmic role may be due to the fact that a comparison of reciprocal androgenetic hybrids and androgenetic controls has not been made. Differences in development which might appear in such a comparison may well be ascribed to the action of the cytoplasm. Suitable for such a study are the two morphologically and physiologically distinct species, *Rana pipiens* Schreber and *Rana palustris* Le Conte. Moore (1941) has shown that reciprocal hybrids of these species develop normally and metamorphose, with the hybrids intermediate in appearance between the two species.

The development of the reciprocal androgenetic hybrids between these two frog species will be described in this paper. Their development will be compared with each other and with their androgenetic controls, with the hope that some information may be obtained on the role of the cytoplasm in early development.

I wish to thank Professor L. G. Barth for his interest and guidance in this work.

Materials and Methods

Eggs and sperm of *R. pipiens* and *R. palustris* were used in these experiments. The eggs were obtained by either pituitary induced ovulation or from females col-

[1] Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University.

lected in the breeding season with the eggs in the oviducts. Sperm were obtained by cutting up the testes in a small quantity of spring water. Approximately 20 minutes after fertilization the eggs were examined to see if the region where the second polar body comes off was visible. This region, which is called the "Keimpunkt" or "Eispek" by the German authors, is a very small, round, dark indentation on the surface of the animal pole and marks the position of the second maturation division spindle (Fig. 1). Using the method of Porter (1939), the maternal chromosomes were removed by inserting the tip of a sturdy glass needle under the Eispek and lifting the second maturation division spindle out in a small exovate. The eggs subsequently cleaved with only the paternal chromosomes. This method of removal of the maternal chromosomes is successful 97 per cent of the time. The removal of a small exovate does not injure the egg nor alter its later development. This was tested by removing a small amount of protoplasm a short distance from the Eispek. No effect on subsequent development was noted. At the early blastula stage the androgenetic embryos, marked by the small exovate, were separated from the diploid embryos. Only blastulae in which the operation

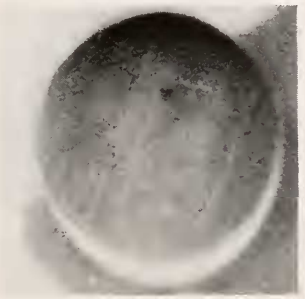


FIGURE 1. The egg of *Rana palustris* 20 minutes after fertilization, showing the small, dark Eispek at the animal pole.

had been well performed were maintained. Approximately equal numbers of diploid blastulae were kept as controls. The embryos were raised in a 12° C. cold room.

The embryos were fixed in Smith's fluid, sectioned at 10 micra, stained with the "Nuclealfarbung" method of Feulgen, and counterstained with light green (Moore and Ryan, 1940). The sectioned embryos were studied and chromosomal counts made. To check on the interpretations, certain embryos were restained with a triple stain recommended by Dr. S. M. Rose. This consisted of picric acid to stain the yolk and endoderm, blau schwartz to stain cell walls and ectoderm, and Orange G to stain both cytoplasm and mesoderm. The stages used to describe the development of the embryos are those of Pollister and Moore (1937).

In referring to individuals, the specific names will be shortened to *pip* and *pal*. The first name will indicate the egg, and the second name the sperm. If the maternal chromosomes were removed, the first name will be in parentheses, indicating a haploid embryo. For example, pip pal means that a pipiens egg was fertilized by a palustris sperm, resulting in a diploid hybrid. In a (pip) pal embryo

the maternal pipiens chromosomes have been removed, resulting in an androgenetic embryo with palustris chromosomes in pipiens cytoplasm.

In a study of androgenetic hybrid embryos, several controls are needed. These controls are the normal diploid embryos, the diploid hybrid embryos and the androgenetic embryos. In order to simplify the description of the experimental material, this material will be divided into two parts. The first part will be a description of those embryos in which the egg of *R. pipiens* was used, and the second part will deal with those embryos in which the egg of *R. palustris* was used.

The Development of (pip) pal Embryos and Their Controls

In this experiment eggs obtained from one Vermont pipiens were used. Some of the eggs were fertilized by sperm from one Vermont pipiens. The remainder of the eggs were fertilized by the combined sperm of two palustris from Woods Hole, Mass. The Eispek was removed from some of the eggs. A total of 204 (pip) pal, 112 (pip) pip, 145 pip pal and 40 pip pip embryos were made and studied. The results from this one large experiment were similar to preliminary experiments when four different pipiens females and five different palustris males were used.

Since the diploid embryos pip pip and the diploid hybrids pip pal were perfectly normal, no description is needed of their development. However, the androgenetic control embryos (pip) pip develop abnormally and a brief description, based on my material, follows. Porter (1939) has described the development of androgenetic pipiens in detail.

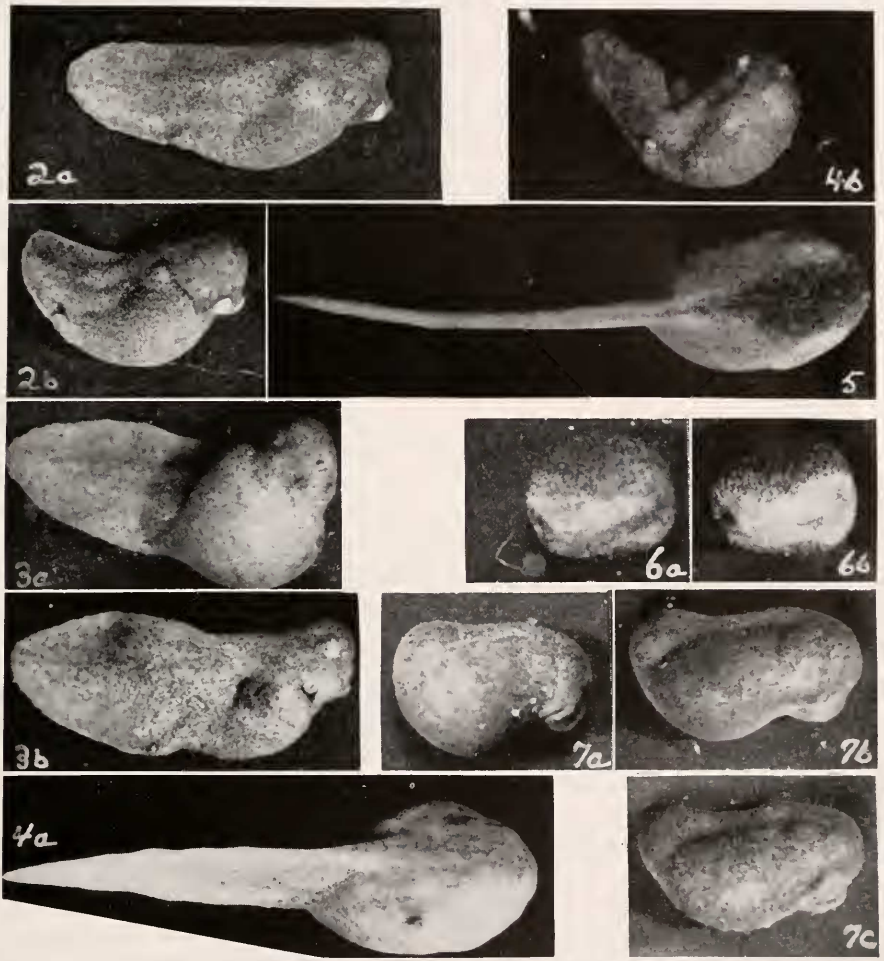
Androgenetic pipiens eggs cleave and gastrulate normally. Their development is delayed during gastrulation. A few embryos begin to cytolyze during the neural fold stage. At the time of closing neural folds many haploids have smaller neural folds than the diploids, and are retarded in development. When the diploid controls pip pip reach stage 19 (heart beat), the haploid (pip) pip are in stage 18 (muscular contraction). When the diploid controls reach stage 20 (gill circulation), the haploids resemble a late stage 18. A few show heart beat and tiny gill projections, but the remainder show neither. All have very large pronephric swellings, wrinkled abdominal epidermis, deep stomadeal pit and a short, broad body (Figs. 2a and 2b). When the diploid controls reach stage 22 (perforated mouths and with the gills almost overgrown by the operculum), the haploids have an open mouth and a small operculum growing over the tiny gill projections. Some of the haploids are very swollen and bloated in appearance, and some are collapsed and wrinkled. When the controls are in stage 23 (horny teeth and with the operculum completely covering the gills), most haploids show no further differentiation than described above. They are either swollen or collapsed and appear to be dying (Figs. 3a and 3b).

The percentage of haploid survival is very high up to tailbud stage. After this stage many embryos begin to die, and differentiation is retarded and incomplete, even though survival may continue for some time.

From the foregoing description it is evident that haploid development is distinct from diploid development. Haploids are easily recognized by their characteristic development, which is retarded and abnormal to a varying degree.

The development of the androgenetic hybrid embryos (pip) pal was markedly different from the control haploid pipiens development. The embryos were re-

tarded during gastrulation so that when the control pip pal embryos had reached an early yolk plug stage, these embryos still had a semicircular dorsal lip. When the (pip) pal embryos reached an early yolk plug stage, some of the yolk cells appeared vacuolated. A small normal yolk plug was formed in all, and up to this time no cytolysis had occurred. By the time the control hybrids were in an early neural fold stage, cytolysis of the haploid hybrids began. The (pip) pal formed a flat



FIGURES 2a and 2b. Androgenetic pipiens embryos whose diploid controls are in stage 20.
FIGURES 3a and 3b. Androgenetic pipiens embryos whose diploid controls are in stage 23.
FIGURES 4a and 4b. Androgenetic pipiens embryos showing the degree of variation in differentiation. Embryos a and b are the same age and from the same experiment.
FIGURE 5. The diploid pipiens control embryo for Figures 4a and 4b.
FIGURE 6a. Elongated (pip) pal neural plate embryo.
FIGURE 6b. A (pip) pal embryo showing the exposed gray cells of the neural plate region.
FIGURE 7. Control embryos for the (pip) pal embryos figured in 6a and 6b. a, (pip) pip. b, pip pip. c, pip pal.

neural plate, and the embryo elongated slightly (Fig. 6a). In those embryos in which the yolk plug was still evident, the yolk plug cells had begun to cytolyze. The wave of cytolysis spread anteriorly, eventually involving the whole neural plate. In embryos in which the yolk plug was a mere slit, a few loose cells appeared on the posterior edge of the neural plate, and again the wave of cytolysis progressed anteriorly. When the vitelline membrane was removed from an embryo, these loose cells came off with it. This revealed the neural plate region as a mass of gray cells, the pigmented ectodermal cells having been sloughed off (Fig. 6b). These (pip) pal embryos can be compared with their controls in Fig. 7.

The (pip) pal embryos were still neural plate embryos when pip pal had closed neural folds. Only 20 (pip) pal survived until the pip pal had reached a tailbud stage. Of these, 3 were still neural plate embryos, 13 showed very slight neural folds and 4 were normal diploid embryos. No further differentiation of (pip) pal embryos occurred, and death followed shortly. This retardation and stoppage of development of (pip) pal embryos is shown in Table I.

TABLE I

A record of the stage of development of (pip)pal embryos and their controls on successive days

Age in days	The stage of development of			
	(pip)pal	(pip)pip	pip pal	pip pip
5th day	11	11–12	12	12
6th day	12	12	12	12–13
7th day	12	12–14	13	13–14
8th day	12–13	13–16	14–15	15–16
9th day	13	14–17	14–16	16–17
10th day	13–14	16–17	16–17	17

Seven (pip) pal embryos were sectioned, varying from a neural plate to a faint neural fold stage, the latter representing the maximum development. A description of these embryos follows after a description of the normal picture in a diploid early neural fold embryo of *R. pipiens*.

A normal embryo is diagrammed in Figure 8. The neural ectoderm is greatly thickened in the anterior and middle regions where it will give rise to brain and neural folds. The notochord in the anterior region is not differentiated. In progressively more posterior sections it gradually becomes differentiated from the mesoderm until it is completely separated from all three layers. The mesoderm in the anterior region continues dorsally as chorda-mesoderm, and extends laterally and somewhat ventrally. Toward the posterior region the mesoderm is completely separated from the notochord and extends a short way laterally. The gut anteriorly has a large lumen which is not roofed by endoderm. Its lumen gradually becomes smaller and completely roofed by endoderm in the posterior region. A liver is present. There are very few cells in mitosis, and no pycnosis nor abnormal nuclei are apparent.

The (pip) pal neural plate embryo is strikingly different. The following description of embryo (pip) pal No. 8, Figure 17, is typical. The neural ectoderm

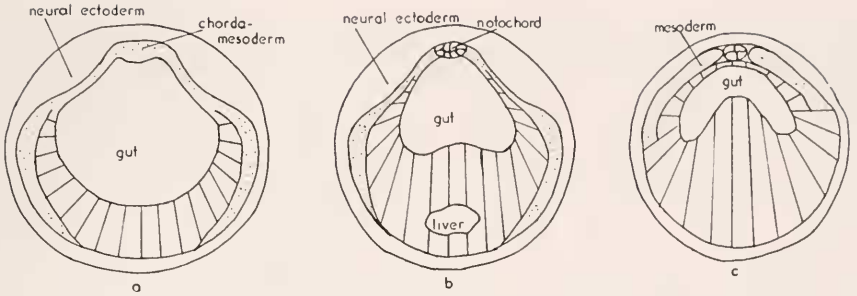


FIGURE 8. Diagram of a diploid pipiens early neural fold embryo, showing an anterior section in a, a middle section in b, and a posterior section in c.

is greatly thickened in the anterior and middle regions of the embryo. Pycnotic nuclei are present in the anterior neural ectoderm which gives rise to brain. In the most anterior region the pycnotic nuclei number about 20 per section. They decrease in number until, in approximately the region where brain and neural tube would join, none are present. In some of these nuclei the chromatin is clumped together. In others the center of the nucleus is faintly stained, and around the periphery is heavily stained chromatin. Still other nuclei are small, condensed and heavily stained.

In the anterior region of the embryo the mesoderm is first distinguishable as a separate layer laterally. It continues dorsally where it fuses with the ectoderm and endoderm. There is no differentiation into chorda-mesoderm. Near the middle of the embryo the mesoderm is separate from the ectoderm and endoderm dorsally and extends laterally and somewhat ventrally. Toward the posterior part of the embryo the mesoderm is a complete layer ventrally as well as dorsally. No notochord is present here or anywhere thoroughout the whole embryo.

The gut along its whole length has a very small lumen which is roofed with endoderm. No liver is present. Both the extent of the mesoderm and the roofing of the gut are characteristic of older diploid closed neural fold embryos, not of neural plate embryos. In contrast to a normal diploid neural plate embryo, many

TABLE II

A comparison of the developmental features of each sectioned (pip)pal embryo with a normal diploid neural plate embryo. The lengths of various organs are measured in micra from the anterior end of each embryo

Embryo	Total length	Gut extends from	Total length of gut	Notochord extends from	Liver extends from
pip pip	1730	130–1510	1390	640–1420	350–650
(pip)pal No. 1	1770	380–1320	950	0	0
(pip)pal No. 2	1890	140–1510	1380	0	0
(pip)pal No. 3	1840	220–1640	1430	0	0
(pip)pal No. 4	1740	180–1470	1300	0	0
(pip)pal No. 6	1700	190–1360	1180	0	0
(pip)pal No. 7	1840	250–1720	1480	0	0
(pip)pal No. 8	1710	80–1500	1430	0	0

cells of the ectoderm and mesoderm were in mitosis. Chromosomal counts showed all 7 embryos to be haploid, with 13 chromosomes. In Table II the developmental features of (pip) pal embryos are summarized and compared with a normal pipiens neural plate embryo.

Embryo (pip) pal No. 6 differed from the other embryos sectioned in that the neural plate cells had cytolyzed. A cross section of this embryo is shown in Figure 18. The dorsal ectoderm is a mass of loose, spongy cells with poorly staining cytoplasm and with condensed, pycnotic nuclei. Many of the ectodermal cells have been sloughed off, as no surface coat is present at the outer surface of the loose cells. The whole length of the neural plate shows cytolyzing cells. From one-quarter to one-third of the way from the anterior end of the embryo, all of the dorsal ectodermal layer and possibly some of the underlying mesoderm is involved in this cytolysis. This is difficult to ascertain, as in this region mesoderm and ectoderm are difficult to separate dorsally. More posteriorly, only the ectoderm is involved. Very few cells of this embryo were in mitosis. In Figure 17 the cytoplasm of the neural ectodermal cells is poorly stained and seems altered in character. This change may well presage the sloughing off of these cells as pictured in Figure 18. Embryo (pip) pal No. 1 also showed cytolysis of the dorsal ectodermal cells, but to a minor extent.

From the foregoing description of (pip) pal embryos it is evident that gastrulation is accomplished, and the three germ layers are formed. Neither notochord nor liver differentiate. The embryos remain in an arrested abnormal neural plate stage. Pycnotic nuclei are present in the neural ectoderm which will give rise to brain. Mitotic activity ceases. The ectodermal cells at the dorsal edge of the blastopore round up and begin to slough off. This cytolysis of the ectodermal cells spreads anteriorly until the whole neural plate is involved, and the embryo then dies.

The Development of (pal) pip Embryos and Their Controls

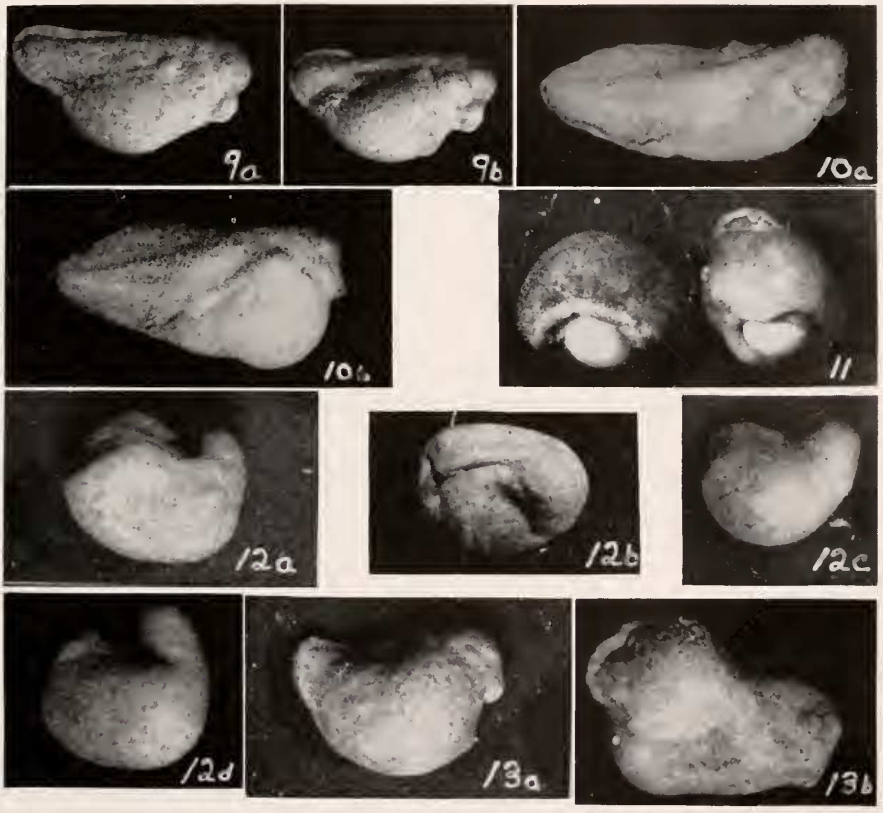
In this cross, 3 experiments were performed at different times. In each experiment eggs were obtained from one palustris female and part of the eggs were fertilized by a palustris male and the remainder by a pipiens male. Thus three different palustris females, 4 palustris males (the combined sperm of two small males were used in one experiment) and 3 pipiens males were used. A total of 304 (pal) pip embryos, 148 (pal) pal, 363 pal pip and 214 pal pal were made and studied.

The diploid embryos pal pal and the diploid hybrids pal pip developed perfectly normally. The (pal) pal embryos were similar in their development to (pip) pip embryos. Figures 9 and 10 show haploid palustris tadpoles. They have short, broad tails, large swollen bellies, and microcephalic heads with optic vesicles, olfactory pits and suckers. The (pal) pal embryos differ from (pip) pip in that their survival to later stages (Table IV) and their degree of differentiation is low. Their maximum development is shown in Figure 10. Only 4 of the 148 embryos survived until the control palustris diploids were in stage 21 (cornea transparent). In these (pal) pal the gills developed as a tiny, short filament, the stomadeum was a very small opening, and the heart was observed beating.

The androgenetic hybrid embryos (pal) pip develop normally up to gastrula-

tion. From the beginning of gastrulation their development is retarded, abnormal and highly variable. The dorsal lip may remain as a crescent and a small neural plate may appear and two small tailbuds may project above the large yolk plug (Fig. 11). These embryos differentiate no further, though they may survive until their controls (pal) pal are in stage 18 and pal pip and pal pal in stage 19. In some embryos a large yolk mass and short anterior projection are present (Fig. 12a).

A few embryos formed dorsal and lateral lips of the blastopore, although no ventral lip was present. In these embryos invagination and overgrowth may occur to such an extent that only a tiny yolk plug is left. Low neural folds may form and close (Fig. 12b) and an anterior projection may develop upon which no morphological head structures are discernible (Figs. 12c and 12d). The percentage of



FIGURES 9a, 9b, 10a and 10b. Androgenetic palustris embryos.

FIGURE 11. Two (pal) pip No. 149 embryos showing abnormal gastrulation.

FIGURES 12a and 12b. Two (pal) pip No. 184 embryos, b showing neural folds.

FIGURES 12c and 12d. Two (pal) pip No. 273 embryos, showing anterior projection and short tail.

FIGURES 13a and 13b. Two (pal) pip No. 184 embryos, showing maximum differentiation reached by any (pal) pip embryo.

TABLE III

A record of the percentage of (pal)pip to reach various degrees of differentiation in the different experiments

	Original number	Percentage of (pal)pip showing				
		Incomplete gastrulation	Complete gastrulation	Neural plate	Neural folds	Anterior projection
Exp. 4/24	34	97.1	0.0	—	8.8	0.0
Exp. 4/19	149	98.6	0.0	57.2	18.8	4.5
Exp. 5/2	121	88.2	10.8	66.7	47.7	12.6
Total	304	92.6	4.1	61.4	29.1	7.0

(pal) pip embryos to reach these various degrees of differentiation are shown in Table III.

A few embryos gastrulated completely, but no differentiation of head structures on the anterior projection occurred. Only two (pal) pip out of all 304 embryos showed greater differentiation (Figs. 13a and 13b). Optic vesicles and suckers are recognizable in one. The percentage of survival of (pal) pip embryos is lower than that of (pal) pal and no (pal) pip embryo survived longer than control stage 19 (Table IV). The retardation of development of these androgenetic hybrid embryos is marked and is summarized in Table V.

Fifteen (pal) pip embryos were sectioned. Thirteen of these ranged in development from an early neural plate stage with a large yolk mass to a closed neural

TABLE IV

A comparison of the percentage of survival of (pal)pip embryos and their controls at various stages of development

	Original number	Percentage of survival when the control pal pip were in		
		Stage 13 6th day	Stage 17 9th day	Stage 18–19 12th day
(pal)pip	304	90.6	31.2	11.1
(pal)pal	148	92.1	40.9	20.4
pal pal	214	100.0	99.6	93.6
pal pip	363	98.2	95.0	93.4

TABLE V

A comparison of the stages of development of pal pip and (pal)pip embryos

pal pip	9	10	11	12	14–15	16	17	18–19
(pal)pip	9	10	10	11	11 with neural plate	11 with neural folds	11 with closed neural folds	11 with anterior projection or closed blastopore with projection

fold stage with an anterior projection. Two were the embryos which have been described as the most advanced. The presence or absence of various organs and their lengths are recorded in Table VI, where they are also compared with data from normal pip pip embryos. In addition, each embryo has been graphically reconstructed in Figures 14, 15, and 16. Each embryo is identified by a number. Those with the same initial numbers, for example 250–1 and 250–2, are from the same experiment and fixed at the same time, so that they are of the same age,

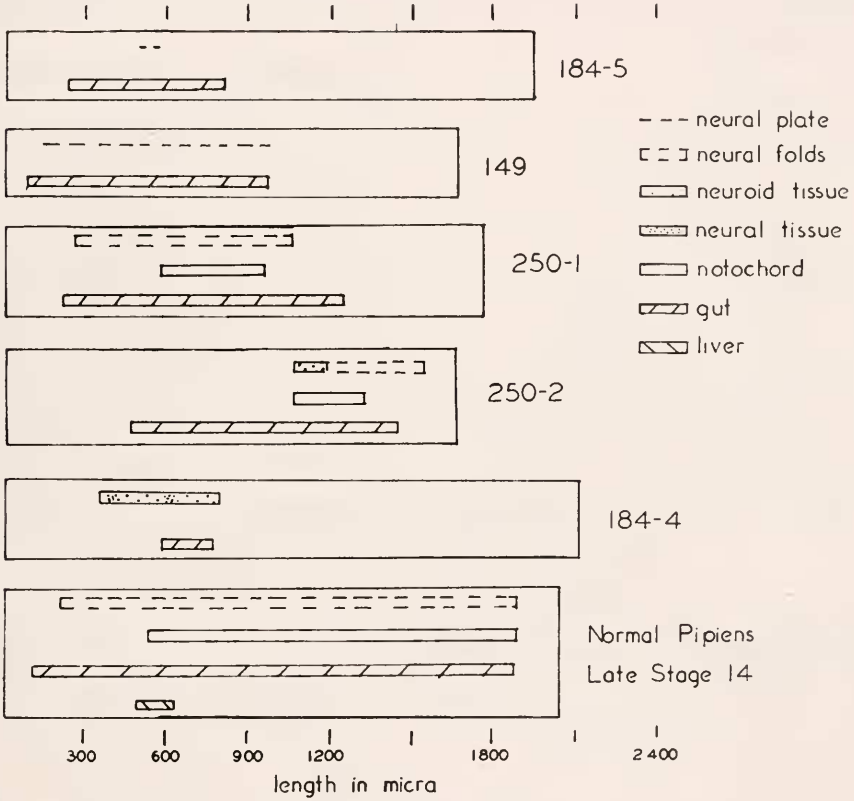


FIGURE 14. Graphic reconstructions of 5. (pal) pip embryos and of a normal late stage 14 pip pip embryo No. 168.

though of various degrees of differentiation. Some of the sectioned embryos with the initial numbers 149 have been shown as whole embryos in Figure 11, 273 in Figures 12c, 12d and 184 in Figures 12a, b and 13a, b.

The five least differentiated (pal) pip embryos are graphically reconstructed in Figure 14 and compared with a normal pip pip late stage 14 (neural folds) whose stage of development they most closely resemble. Their control pal pip embryos ranged in age from stage 14 for embryo 149 to stage 16 (closed neural folds) for embryos 250–1 and 250–2, to stage 19 (heart beat) for embryos 184–5 and 184–4.

A large yolk mass protruded dorsally in the posterior half of each embryo except 250–2.

The five (pal) pip embryos which survived until the control pal pip were in either stage 18 (muscular contraction) or stage 19 are graphically reconstructed in Figure 15, and compared with a normal pip pip early stage 16 embryo whose stage of development they most closely resemble. Each embryo had closed neural folds but a large yolk mass protruded dorsally in embryos 184–8C, 184–7B and 238.

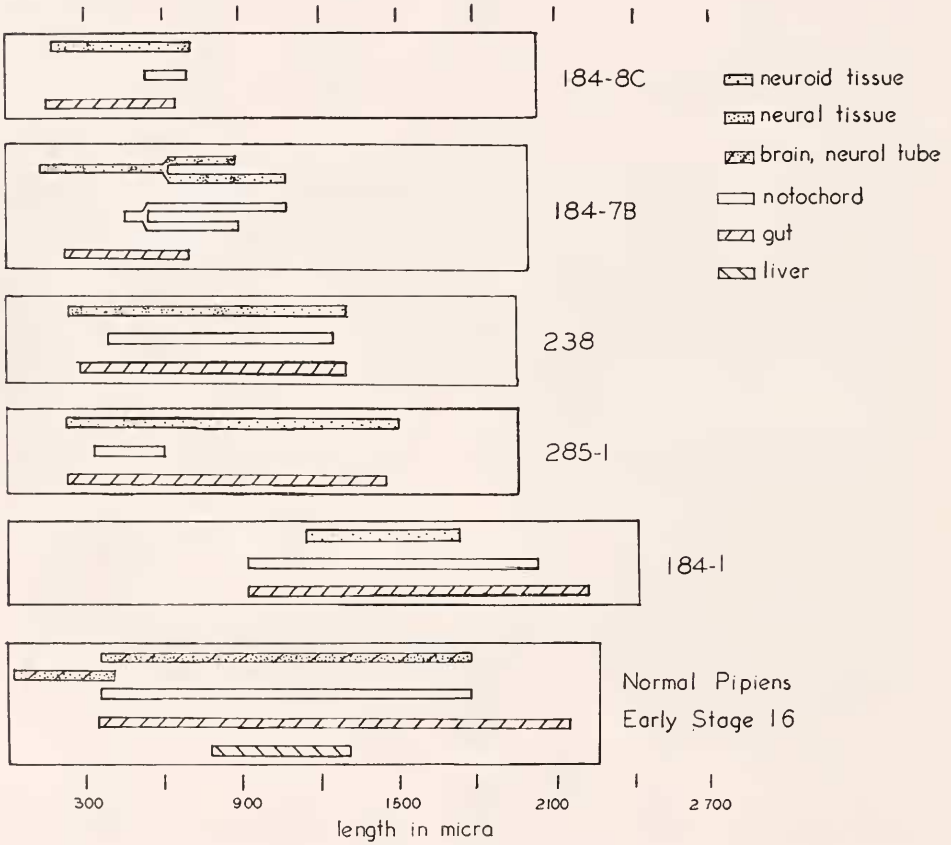


FIGURE 15. Graphic reconstructions of 5 (pal) pip embryos and of a normal early stage 16 pip pip embryo No. 166.

The remaining five (pal) pip embryos also survived until the control pal pip embryos were in either stage 18 or stage 19, but showed greater differentiation. These embryos are graphically reconstructed in Figure 16 and compared with a normal pip pip stage 18 embryo, whose stage of development they most closely resemble. Anterior projections were present in embryos 184–2, 273 and 285–2. Embryos 184–6a and 184–9 were the most advanced (pal) pip embryos shown in Figures 13a and b.

A general description of the internal differentiation of (pal) pip, based on these fifteen sectioned embryos, will now follow.

A neural plate with lateral ectodermal thickenings may differentiate, even though a large yolk plug may be present due to incomplete gastrulation (Fig. 14, embryos 184–5, 149). Neural folds may develop (Fig. 14, embryos 250–1, 250–2) but are very low, with the cells poorly oriented (Fig. 19), contrasting markedly with a normal closing neural fold embryo (Fig. 20). Where these neural folds close, differentiation into neuroid tissue may occur (Fig. 14, embryo 250–2; Fig. 15, embryo 184–1). The term "neuroid tissue" is used to describe a mass of darker

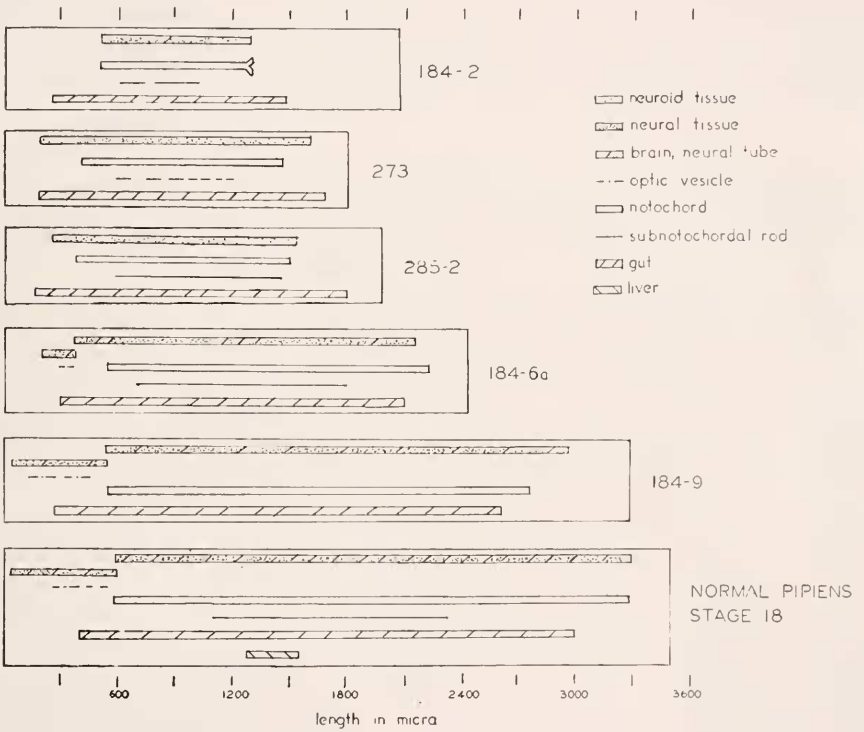


FIGURE 16. Graphic reconstructions of 5 (pal) pip embryos and of a normal stage 18 pip pip embryo No. 613.

staining, poorly differentiated ectodermal cells with scattered pigment throughout. Such neuroid tissue is shown in Figure 21 and can be compared with a normal neural tube in Figure 22.

In some embryos not only is neuroid tissue present, but also neural tissue. The term "neural tissue" is used to define a group of ectodermal cells which are more or less delimited from the surrounding cells by a membrane, and may or may not show a neurocoel (Fig. 23). It is markedly different from a normal neural tube (Fig. 22). Both neuroid and neural tissue are present in varying amounts in the embryos (Figs. 14–16 and Table VI).

In those embryos in which an anterior projection is formed there is a small amount of neuroid tissue and a large amount of neural tissue (Fig. 16, embryos 184–2, 273 and 285–2). The neural tissue may, at times, closely approximate a neural tube (Fig. 26) and in one such embryo (184–2) a neural tube was present for a short distance. This neural tube is thick and abnormal in appearance (Fig. 27) when it is compared with a normal pipiens neural tube (Fig. 28).

Neural tissue, neural tube, brain and optic vesicles are present in the two most advanced embryos (Fig. 16, embryos 184–6a and 184–9). The brain and optic vesicles are very thick walled and abnormal in appearance (Fig. 29) as can be seen by a comparison with a normal embryo (Fig. 30). One of these embryos (184–9) had a thick walled hind brain and paired otic vesicles (Fig. 31) markedly different from those in a normal embryo (Fig. 32). Its neural tube was very abnormal posteriorly, appearing as a round vesicle with a single layer of cells (Fig. 33).

TABLE VI

A comparison of the lengths of organs found in each sectioned (pal)pip embryo with those of normal pip pip embryos. Length is in micra. c = control pal pip. st = stage of development

(pal)pip embryo number	Total length	Length of								
		Neural plate	Neural folds	Brain	Neuroid and neural tissue	Neural tube	Notochord	Subnoto-chordal rod	Gut	Liver
184–5 c-st 19	1960	80					0		570	0
149 c-st 14	1670	830					0		890	0
250–1 c-st 16	1770		790				400		1010	0
250–2 c-st 16	1670				480		240		980	0
184–4 c-st 19	2120				400		0		170	0
184–8c c-st 19	2050				530		170		500	0
184–7b c-st 19	2010				760 / 470 \ 940		440		480	0
238 c-st 19	1970				1070		860		1030	0
285–1 c-18	1970				1290		270		1240	0

TABLE VI.—*Continued*

(pal)pip embryo number	Total length	Length of								
		Neural plate	Neural folds	Brain	Neuroid and neural tissue	Neural tube	Notochord	Subnoto-chordal rod	Gut	Liver
184–1 c-st 19	2440				580		1130		1310	0
184–2 c-st 19	2080				780		800 770 800	350	1230	0
273 c-st 18	1810				1430		1070	510	1490	0
285–2 c-st 18	1970				1290		1120	880	1640	0
184–6a c-st 19	2440			170	1780		1690	1120	1820	0
184–9 c-st 19	3290			500		2430	2230	0	2350	0
pip pip 168 st 14	2050		1700				1380		1680	140
pip pip 166 st 16	2250			410		1510	1510		1870	290
pip pip 613 st 18	3540			570		2730	2730	1240	2610	270

A notochord was absent in the (pal) pip neural plate embryos and in one closed neural fold embryo (Fig. 14, embryo 184–4). The notochord was present in the remaining closed neural fold embryos but it was highly variable in length and bore no relationship to the length of the neuroid and neural tissue (Figs. 14 and 15). In the most highly defferentiated (pal) pip embryos (Fig. 16) a well developed notochord and subnotochordal rod were present (Fig. 26).

A gut with a small lumen was present in all embryos. No liver was present in any (pal) pip embryos. A mesodermal layer was absent in the most poorly differentiated embryo 184–5, but present in all the rest of the embryos of Figs. 14 and 15 and embryo 285–2 of Fig. 16. The remaining embryos of Fig. 16 showed somites. A short pronephric duct was present in the most advanced embryo (184–9). A heart was not present in any (pal) pip embryo.

Chromosomal counts showed the following embryos to be haploid: 184–5, 250–2 and 184–4 of Figure 14; 184–7B and 285–1 of Figure 15; 273 and 184–6a of Figure 16. The remaining 8 (pal) pip embryos were either mitotically inactive or with nuclei so faintly stained that chromosomal counts could not be made. They

are believed to be haploid as their differentiation was similar to other (pal) pip embryos in which chromosomal counts could be made.

Pycnotic nuclei and loose cells were noted in some of the least differentiated and in some of the most highly differentiated embryos. Figure 24 shows such loose ectodermal and mesodermal cells which were scattered in a large fluid filled space about the notochord of embryo 184–1, Figure 15. The pycnotic nuclei (Fig. 25) of these loose cells show either peripheral clumping of the chromatin or they are small, condensed and darkly staining. Pycnotic nuclei were present in ectodermal, mesodermal and endodermal cells (embryo 184–6a, Fig. 16).

Oedema occurred in some of the less differentiated as well as in some of the most highly differentiated embryos. The oedematous spaces were within the ectoderm, between the ectoderm and endoderm, or between the ectoderm and mesoderm. One such large space is shown in Figure 33.

From the foregoing description it is evident that even though gastrulation in (pal) pip embryos may be incomplete, a neural plate with lateral ectodermal thickenings is formed. A shallow neural groove and low neural folds with poorly oriented ectodermal cells is then formed. When the folds close, neuroid and neural tissue are formed. With more complete gastrulation, differentiation into a recognizable neural tube and even into brain may occur. A notochord develops. A gut but no liver is present. A mesodermal layer and even somites are present. Oedema may occur. Pycnosis may be associated with the breakdown of tissues.

Discussion

The discussion will be devoted to the consideration of haploidy in amphibians and of the results obtained by combining a haploid set of chromosomes of one species with the cytoplasm of a different species.

The abnormal development of haploid parthenogenetic and androgenetic amphibian embryos has been ascribed to the condition of haploidy *per se* or to the presence of unmasked lethal or semi-lethal genes. It would seem logical that both factors play a role. A characteristic haploid syndrome may be present. Haploid tadpoles are usually small, with short bodies, broad tails, abnormal eyes, a poorly looped gut and oedema. The degree of differentiation is, however, highly variable. In a large group of (pip) pip embryos the degree of abnormality may vary from extreme to slight. Two such embryos are shown in Figures 4a and

Plate I

Figure 17. Section of embryo (pip) pal No. 8, 610 micra from anterior end, showing thickened neural ectoderm.

Figure 18. Section of embryo (pip) pal No. 6, 920 micra from anterior end, showing cytolyzing neural plate cells.

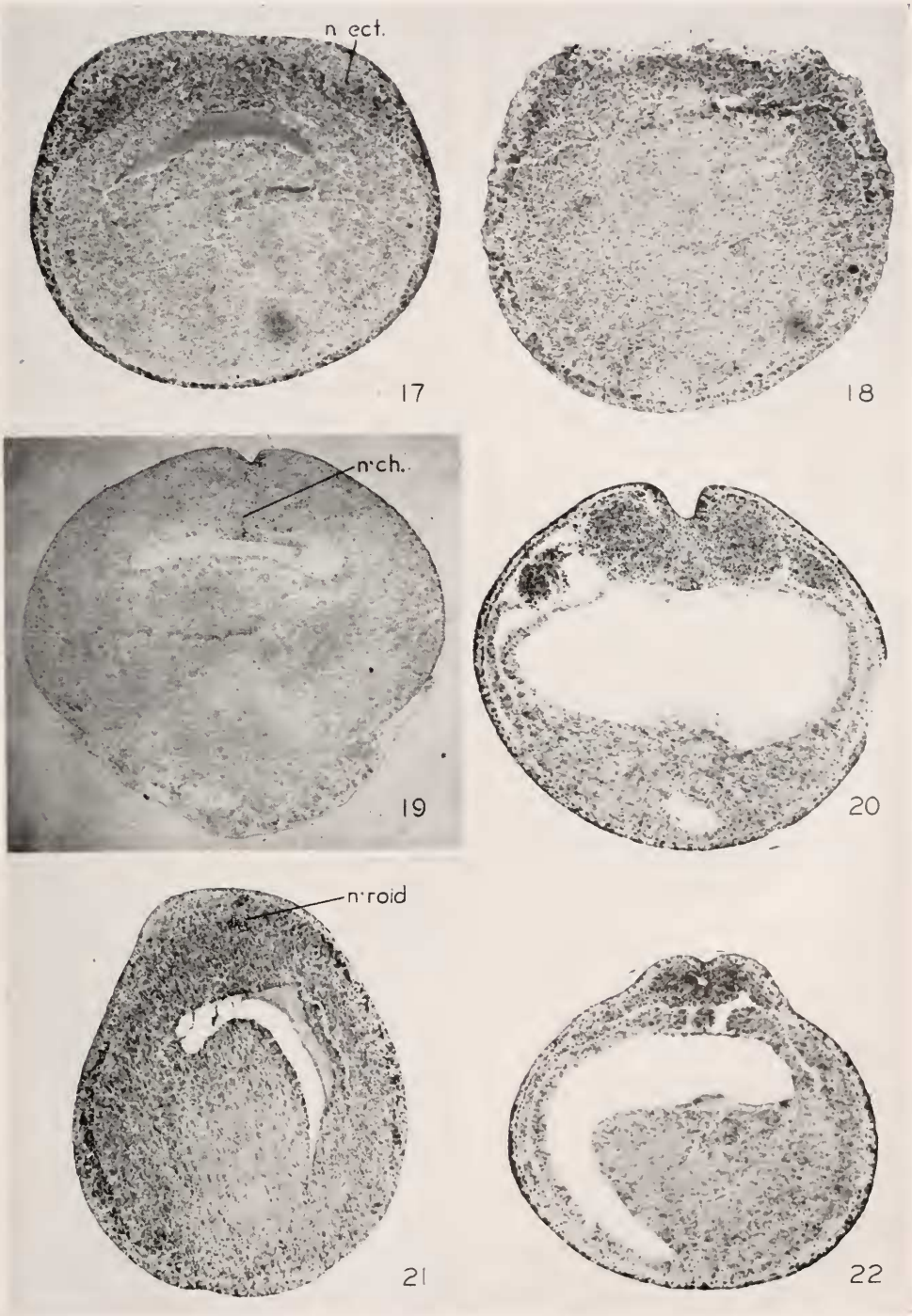
Figure 19. Section of embryo (pal) pip 250–1, 750 micra from anterior end, showing neural folds.

Figure 20. Section of embryo pip pip 168, 530 micra from anterior end showing normal neural folds.

Figure 21. Section of embryo (pal) pip 250–2, 1100 micra from anterior end, showing neuroid tissue.

Figure 22. Section of embryo pip pip 166, 830 micra from anterior end, showing normal neural tube.

PLATE I



b, which can be compared with their normal control in Figure 5. Such variations among haploid Triturus larvae have been described by Baltzer (1922), Baltzer and de Roche (1936) and Fankhauser (1930). These *variations* of development, superimposed on the haploid syndrome, are probably due to the different genetic constitution of each embryo. In the haploid condition recessive genes are unmasked. If any of these are embryonic lethals or semi-lethals, their effects will be observed in development. In haploid embryos in which the majority of the genes are of the normal type, it is possible that differentiation may go far and that even metamorphosis may be reached. This view is supported by the fact that Baltzer (1922) and Fankhauser (1938) described a metamorphosed haploid *T. taeniatus*. With increasing numbers of semi-lethal and lethal genes, haploid differentiation would become poorer and death occur at earlier stages. Thus genetic differences would explain the variability of haploid development.

As evidence against this hypothesis the viability of diploid parthenogenetic frogs (Parmenter, 1933) has been cited. If these diploids were produced by a doubling of the chromosomes at the first mitotic cleavage, completely homozygous individuals would result. Such individuals, homozygous for semi-lethals and lethals, should be inviable. As a matter of fact, only half of the few parthenogenetic diploids of Parmenter metamorphosed, the remainder dying at an early stage. Kawamura (1939) found that in a large number of diploid parthenogenetic frogs only 17 per cent metamorphosed, and even these showed slight irregularities. The remaining diploids were abnormal and his drawings of some of these diploids markedly resemble embryos with the haploid syndrome. Such results tend to support the hypothesis that unmasked recessive genes play a role in abnormal haploid development. Those embryos which have unmasked lethals and semi-lethals can not survive in either the haploid or diploid condition.

In those parthenogenetic cases where diploidy is restored by the retention of two sets of maternal chromosomes through the suppression of a meiotic division, a certain degree of heterozygosity would result and such diploids might have normal viability.

The injurious effect of haploidy varies from species to species in amphibia. In my experiments, haploid pipiens embryos live longer and differentiate further than haploid palustris embryos. Androgenetic *T. taeniatus* have reached metamorphosis (Baltzer, 1922), whereas androgenetic *T. palmatus* and *alpestris* survive only until limb bud stages (Fankhauser, 1930; Baltzer and de Roche, 1936). Haploid *R. esculenta* (Dalcq, 1932) usually form neurulae, and some develop tiny

---

PLATE II

FIGURE 23. Section of embryo (pal) pip 285–1, 430 micra from anterior end, showing neural tissue.

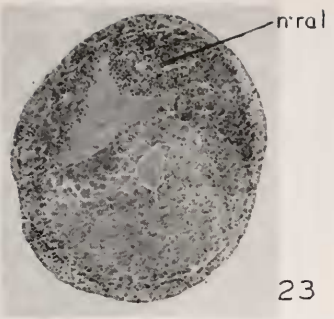
FIGURE 24. Section of embryo (pal) pip 184–1, 1600 micra from anterior end, showing loose cells in fluid filled space about notochord.

FIGURE 25. Pycnotic nuclei present in the loose cells shown in Figure 24.

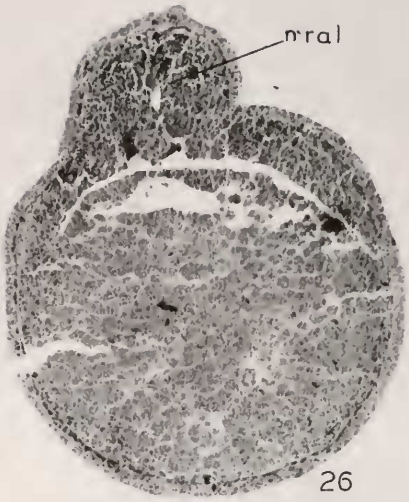
FIGURE 26. Section of embryo (pal) pip 184–2, 960 micra from anterior end, showing neural tissue, notochord, and subnotochordal rod.

FIGURE 27. Section of embryo (pal) pip 184–2, 890 micra from anterior end, showing neural tube.

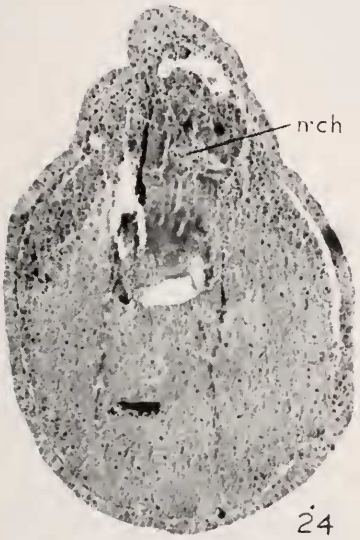
FIGURE 28. Section of embryo pip pip 613, 1270 micra from anterior end, showing normal neural tube.



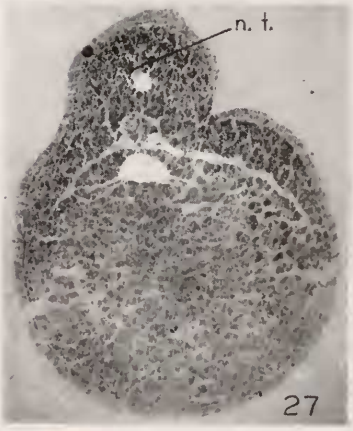
23



26



24



27

25

28

gills. Fankhauser (1937) and Kaylor (1940) found that in haploid *T. pyrroghaster* approximately half of the embryos die as gastrulae but that others go on to a forelimb bud stage. Stauffer (1945) found that androgenetic axolotl survive only as long as the gastrula and neurula stage. Griffiths (1941) showed that the majority of androgenetic *T. viridescens* embryos die as blastulae and gastrulae, but about 10 per cent form neurulae.

Death during late blastula and early gastrula stages is probably not due to haploidy but to abnormal chromosomal distributions and mitotic aberrations. Because of polyspermy in Triturus, a balanced set of chromosomes may not be present and Fankhauser (1934) has pointed out that such a balanced set appears to be needed for gastrulation and later development. Development to a late blastula or early gastrula stage appears to be under maternal cytoplasmic control, and further differentiation is under genic control. Boveri (1907) noted this in dispermic sea urchin eggs and Moore (1941) has discussed this concept in relation to frog hybrids. All of these findings corroborate the probability that the abnormal development of haploid embryos is partially a result of genic action.

From the data presented it is evident that the results obtained by combining a haploid set of palustris chromosomes with the cytoplasm of *R. pipiens* are markedly different from those results when the palustris chromosomes are in their own cytoplasm. These (pip) pal embryos die at an earlier stage than (pal) pal or (pip) pip. Some process or processes in differentiation are either lacking or are unable to carry out their normal functions. For example, the absence of the differentiation of the neural tube may be explained embryologically by several possibilities. (1). The organizer may be in a weakened condition, so that a neural tube can not be induced. (2). The organizer may be normal but the neural ectoderm may be weakly competent and thus no neural tube is formed. (3). The organizer may be weak *and* the neural ectoderm poorly competent, therefore no neural tube is formed. A definite answer to these three possibilities can only be obtained by xenoplastic implants and transplants of the materials involved.

Similarly, the results obtained by combining a haploid set of pipiens chromosomes with the cytoplasm of *R. palustris* are markedly different from those results when the pipiens chromosomes are in their own cytoplasm. These (pal) pip embryos differentiate much more abnormally than (pip) pip or (pal) pal. The neural ectoderm is particularly affected, as a recognizable brain and neural tube are rarely formed. In these embryos, as in (pip) pal, the competence of the neural ectoderm may be lowered or the inductive capacity of the organizer may be weakened,

---

PLATE III

FIGURE 29. Section of embryo (pal) pip 184-9, 210 micra from anterior end, showing brain and optic vesicles.

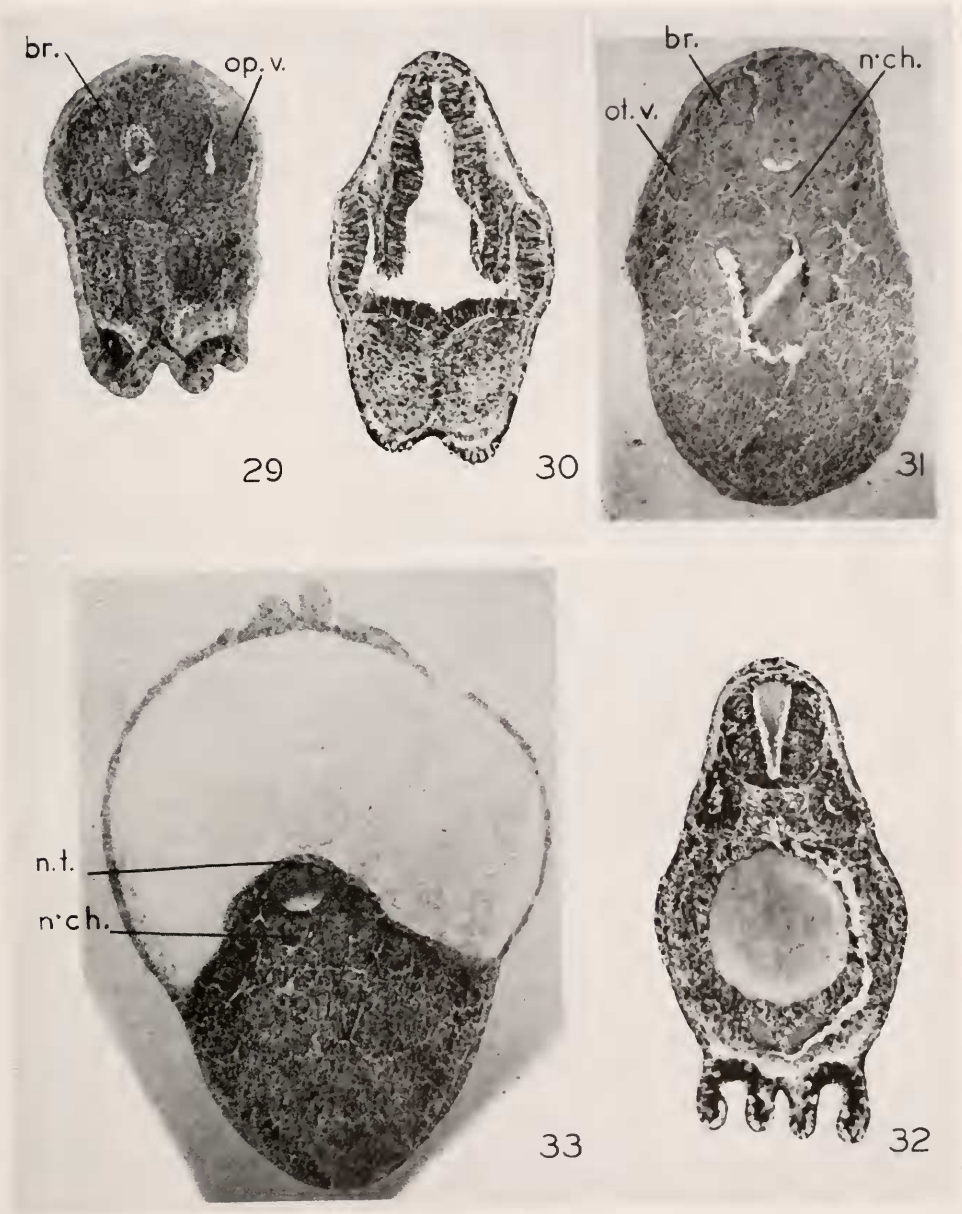
FIGURE 30. Section of embryo pip pip 613, 420 micra from anterior end, showing normal brain and optic vesicles.

FIGURE 31. Section of embryo (pal) pip 184-9, 740 micra from anterior end, showing otic vesicle.

FIGURE 32. Section of embryo pip pip 613, 800 micra from anterior end, showing normal otic vesicles.

FIGURE 33. Section of embryo (pal) pip 184-9, 2350 micra from anterior end, showing abnormal neural tube and large oedematous space.

PLATE III



ABBREVIATIONS FOR PLATES

br., brain
op. v., optic vesicle
ot. v., otic vesicle
n'ch., notochord
n. ect., neural ectoderm
n'ral, neural tissue
n'roid, neuroid tissue
n.t., neural tube

resulting in abnormal development and early death. Both lowered competence and weakened organizer together may play a role. In the (pal) pip neurulae a regional difference in the strength of the organizer may be present, in that the head organizer may be very weak or even absent in most of the embryos. Differentiation of the anterior end of the gut is probably abnormal, since a liver diverticulum never develops. Implantation of the organizer and transplantation of the presumptive neural ectoderm of (pal) pip embryos to a foreign host would clarify the roles they play in the abnormal development of these embryos.

From previous work on amphibian androgenetic hybrids it has long been known that when a haploid set of chromosomes from one species is combined with the cytoplasm of another species, the haploid development differs from that when the chromosomes and cytoplasm are of the same species. For example, Baltzer (1920) has shown that androgenetic *T. taeniatus* embryos form larvae and even reach metamorphosis, but androgenetic hybrids between *T. taeniatus* ♀ X *T. palmatus* ♂ develop only as far as larvae with forelimbs. It is interesting to note that diploid hybrids between these two species are capable of metamorphosing.

From the data presented it is evident that the androgenetic hybrids are alike in showing pycnosis. In (pip) pal embryos the pycnotic nuclei are confined to the neural ectoderm, a region which is mitotically very active in these embryos. In (pal) pip embryos the pycnosis may ultimately involve all tissues but the notochord. The presence of pycnosis has often been described in androgenetic embryos of other amphibia, in androgenetic hybrids and in diploid hybrids. Stauffer (1945) found pycnosis in the blastula and gastrula cells of androgenetic white or black axololts. In the few embryos which formed neurulae he found pycnotic nuclei in the neural tube, brain and mesoderm. Curry (1936) found pycnotic nuclei in the head mesenchyme of the neurulae of androgenetic hybrids between *T. alpestris* females and *T. cristatus* males. He also described the brain lumen as being filled with degenerated cell material. The neural tube was without a lumen and it and the brain were poorly developed. This description of internal development is similar to that of (pal) pip development. Baltzer (1938) described pycnosis in the gastrulae of diploid hybrids between *T. cristatus* females and *T. palmatus* males. Only a few of these hybrids develop as far as neurulae.

From the foregoing it is apparent that pycnosis may be characteristic of tissues which are approaching death and have ceased differentiation and begun to disintegrate. It is a manifestation of abnormal processes which are going on in the embryos. Leuchtenberger (1950) has shown that definite chemical changes are occurring in the nucleus during pycnosis. These changes involve a progessive loss of protein with a depolymerization and progressive loss of the desoxyribose nucleic acid. Thus, during pycnosis, significant chemical changes are occurring in the nuclear material.

From the data presented it is evident that not only do the androgenetic hybrids (pip) pal and (pal) pip differ from their androgenetic controls (pip) pip and (pal) pal, but that they also differ from each other. The haploid set of paternal chromosomes of either pipiens or palustris, when present in its own specific cytoplasm, is capable of normal gastrulation, neurulation and the formation of tadpoles. If pipiens cytoplasm is substituted for palustris cytoplasm, the haploid set of palustris chromosomes is only capable of carrying differentiation to the neural plate stage.

Since the genes in (pip) pal embryos are similar to those in (pal) pal embryos, the difference in the development between (pip) pal and (pal) pal is probably due to differences between pipiens and palustris cytoplasm. In (pal) pal embryos the palustris cytoplasm is able to supply normal materials to the palustris genes for their synthesis. In addition the palustris genes have the palustris cytoplasm to act upon and utilize in differentiation. In the pipiens cytoplasm certain substrates may be the same as those in the palustris cytoplasm, even as certain of their genes are homologous, since the reciprocal diploid hybrids develop normally. (Homologous spotting genes have been shown to be present in pipiens and palustris by Moore, 1943.) Other substrates in the pipiens cytoplasm may be species specific. Baltzer (1930) suggested from his studies of androgenetic hybrids that species specific substances existed in the cytoplasm. The palustris genes may not be able to utilize these species specific substrates, so that development ceases; or they may be able to utilize these substrates in forming compounds which may then act as anti-metabolites (Woolley, 1947), resulting in abnormal development. Similarly, in the reverse cross (pal) pip, the pipiens genes may not be able to utilize certain of the palustris substrates, or these substrates may act as anti-metabolites when utilized, with resulting abnormal development.

Since (pip) pal and (pal) pip embryos differ from each other in their development, different morphogenetic processes are probably upset. The uniformity of (pip) pal development suggests that a certain morphogenetic substance (or substances), which may contribute to one early major developmental step, is lacking or abnormal. For example, such a substance might be involved in the conversion of chordamesoderm to notochord. In this case one might think that the palustris gene controlling this major developmental step is not able to work with the pipiens substrate to form the necessary materials. In the reverse cross, (pal) pip, the great variation in development may indicate that several different substances which contribute to different developmental steps may be lacking or abnormal. In this case one might say that several pipiens genes may be involved in a variety of developmental steps, but are unable to work with the palustris substrates, resulting in abnormal and variable development, since a larger number of different genes and substrates are involved.

Differences between reciprocal androgenetic hybrids and their androgenetic controls have not been previously described and analyzed in detail. Baltzer (1933) has recorded differences between reciprocal androgenetic hybrids of *T. palmatus* and *T. alpestris*. Dalton (1946) noted differences between the reciprocal androgenetic hybrids and androgenetic controls among *T. torosus*, *T. granulosis* and *T. rivularis* embryos but this work dealt primarily with the role cytoplasmic properties may play in transplanted androgenetic hybrid tissue. Dalton found that such transplanted tissue shows the influence of the cytoplasm in the number and dispersion of melanophores. Hadorn (1936) had earlier attempted to show cytoplasmic influence in transplanted androgenetic hybrid epidermis, but with equivocal results.

The results obtained in this study suggest that the abnormal development and death of haploid amphibian embryos is due to both haploidy *per se* and to unmasked lethal and semi-lethal genes. In the development of androgenetic hybrids not only may these two factors be operative but, in addition, the cytoplasmic substrates may play a role.

Summary

The development of androgenetic *R. pipiens* and *R. palustris* embryos has been briefly described. A detailed study of their androgenetic hybrids has been made.

The (pip) pal embryos develop uniformly to an early neural fold stage, then differentiation ceases, the neural plate cells are sloughed off and the embryos die.

The (pal) pip embryos develop uniformly until early gastrulae. Thereafter their development is highly variable. Gastrulation is usually incomplete, and a short neural plate may be formed. In a few embryos closure of the neural folds may occur and neuroid and neural tissue be formed. An occasional embryo is capable of slightly further differentiation, with an abnormal brain and neural tube, and optic and otic vesicles.

The effects of haploidy and the results obtained by combining a haploid set of chromosomes of one species with the cytoplasm of a different species are discussed.

LITERATURE CITED

Baltzer, F., 1920. Über die experimentelle Erzeugung und die Entwicklung von Triton-Bastarden ohne mütterliches Kernmaterial. *Verh. Schweiz. Natf. Ges.*, **101**: 217–220.

Baltzer, F., 1922. Über die Herstellung und Aufzucht eines haploiden Triton taeniatus. *Verh. Schweiz. Natf. Ges.*, **103**: 248–249.

Baltzer, F., 1930. Die Zussamenarbeit von Plasma und Kern in der tierischen Entwicklung. *Mitt. Naturforsch. Ges.* Bern.

Baltzer, F., 1933. Über die Entwicklung von Triton-Bastarden ohne Eikern. *Verh. Dtsch. Zool. Ges.*, **35**: 119–126.

Baltzer, F., 1938. Die Frühenentwicklung der Bastards *Triton cristatus* ♀ × *Triton palmatus* ♂. *Rev. Suisse Zool.*, **45**: 391–394.

Baltzer, F. and V. de Roche, 1936. Über die Entwicklungsfähigkeit haploider *Triton alpestris*-keime und über die Aufhebung der Entwicklungshemmung bei Geweben letaler bastard-merogonischer Kombination durch Transplantation in einer normalen Wirt. *Rev. Suisse Zool.*, **43**: 495–505.

Boveri, T., 1907. Zellenstudien. VI. Die Entwicklung dispermer Seeigeleier. Ein Beitrag zur Befruchtungslehre und zur Theorie des Kernes. *Jen. Z. Naturwiss.*, **43**: 1–292.

Conklin, E. G., 1905. Mosaic development in Ascidian eggs. *Jour. Exp. Zool.*, **2**: 145–223.

Conklin, E. G., 1908. Mechanism of heredity. *Science*, **27**: 89–99.

Curry, H. A., 1936. Über die Entkernung des Triton Eies durch Absaugen des Eifleckes und die Entwicklung des Tritonmerogons *Triton alpestris* (♀) × *Triton cristatus* ♂. *Roux Arch.*, **134**: 694–715.

Dalcq, A., 1932. Contribution à l'analyse des fonctions nucléaires dans l'ontogénèse de la Grenouille. IV. Modifications de la Formule chromosomiale. *Arch. de Biol.*, **43**: 343–366.

Dalton, H. C., 1946. The role of nucleus and cytoplasm in development of pigment patterns in Triturus. *Jour. Exp. Zool.*, **103**: 169–200.

Fankhauser, G., 1930. Entwicklungspotenzen diploidkerniger halften des ungefurchten Tritoneies. *Roux Arch.*, **122**: 671–735.

Fankhauser, G., 1934. Cytological studies on egg fragments of the salamander Triton. V. Chromosome number and chromosome individuality in the cleavage mitoses of merogonic fragments. *Jour. Exp. Zool.*, **68**: 1–58.

Fankhauser, G., 1937. The production and development of haploid salamander larvae. *Jour. Hered.*, **28**: 2–15.

Fankhauser, G. 1938. The microscopical anatomy of metamorphosis in a haploid salamander, *Triton taeniatus* Laur., *Jour. Morph.*, **62**: 393–413.

Griffiths, R. B., 1941. Triploidy (and haploidy) in the newt, *Tr. viridescens*, induced by refrigeration of fertilized eggs. *Genetics*, **26**: 69–88.

HADORN, E., 1936. Übertragung von Artmerkmalen durch das entkernte Eiplasma beim merogonischen Triton-Bastard, palmatus-Plasma × cristatus-Kern. *Verh. Dtsch. Zool. Ges.*, **38**: 97–104.

HERTWIG, P., 1916. Durch Radiumbestrahlung verursachte Entwicklung von halbkernigen Triton-und Fischembryonen. *Arch. mikro. Anat.*, **87**, II: 63–122.

KAWAMURA, T., 1939. Artificial parthenogenesis in the frog. I. Chromosome numbers and their relation to cleavage histories. *Jour. Sci. Hirosima Univ.*, Ser B., Div. 1 (*Zool*), **6** (7/9): 115–218.

KAYLOR, C. T., 1940. Studies on experimental haploidy in salamander larvae. I. Experiments with eggs of the newt, *Triturus pyrrhogaster*. *Biol. Bull.*, **79**: 397–408.

LEUCHTENBERGER, C., 1950. A cytochemical study of pycnotic nuclear degeneration. *Chromosoma*, **3**: 449–475.

MOORE, B. C. AND E. W. RYAN, 1940. Chromosomes of frog eggs and embryos stained by the Feulgen method to avoid excessive staining of yolk granules. *Anat. Rec.*, **78** Suppl. 1: 122.

MOORE, J. A., 1941. Developmental rate of hybrid frogs. *Jour. Exp. Zool.*, **86**: 405–422.

MOORE, J. A., 1943. Corresponding genes in spotted frogs. *Jour. Heredity*, **34**: 3–7.

PARMENTER, C. L., 1933. Haploid, diploid, triploid and tetraploid chromosome numbers, and their origin in parthenogentically developed larvae and frogs of *Rana pipiens* and *R. palustris*. *Jour. Exp. Zool.*, **66**: 409–453.

POLLISTER, A. W. AND J. A. MOORE, 1937. Tables for the normal development of *Rana sylvatica*. *Anat. Rec.*, **68**: 489–496.

PORTER, K. R., 1939. Androgenetic development of the egg of *Rana pipiens*. *Biol. Bull.*, **77**: 233–257.

STAUFFER, E., 1945. Versuche zur experimentellen Herstellung haploider Axolotl-Merogone. *Rev. Suisse Zool.*, **52**: 231–327.

WILSON, E. B., 1904. Experimental studies on germinal localization. I. The germ regions in the egg of Dentalium. *Jour. Exp. Zool.*, **1**: 1–72.

WILSON, E. B., 1904. Experimental studies on germinal localization. II. Experiments on the cleavage—mosaic in Patella and Dentalium. *Jour. Exp. Zool.*, **1**: 197–268.

WOOLLEY, D. W., 1947. Recent advances in the study of biological competition between structurally related compounds. *Physiol Rev.*, **27**: 308–333.